Electronic Supplementary Information:

Electrochemical approach for detection of DNA methylation and assay of the methyltransferase activity

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1. Chemicals.

All chemicals and solvents were of reagent grade or better. Hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$), ferroceneacetic acid (FcA, 98%), tris(hydroxymethyl)aminomethane (Tris), tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 98%), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide (EDC), *N*-hydroxysuccinimide (NHS, 98%), dithiothreitol (DTT), bovine serum albumin (BSA), 5-azacytidine (5-Aza), and 5-aza-2'-deoxycytidine (5-Aza-dC) were purchased from Sigma-Aldrich and used as received. *S*-adenosylmethionine (SAM), *E. coli* CpG methyltransferase M. *Sss*I, and *E. coli* restriction endonuclease *Hpa*II were supplied by New England BioLabs (Ipswich, MA). All solutions were prepared with doubly distilled water.

The synthetic oligonucleotides (S2, and S3) were purchased from Shanghai Sheng-gong Biotechnology Co. (Shanghai, China), and their base sequences are as follows:

thiol-capped single-stranded DNA (probe DNA, S1), 5'-SH-$(CH_2)_6$-TTC TCT TCC TCT GTG CGC CGG TCT CTC CCA GG-$(CH_2)_6$-NH_2-3';

one–base mismatched DNA (S3), 5'- CCT GGG AGA GAC C***T***G CGC ACA GAG GAA GAG AA-3'.

137-base sequence (S2) in the promoter region (exon 8) of the Homo sapiens p53 gene, 5'-TGG TAA TCT ACT GGG ACG GAA CAG CTT TGA GGT GCG TGT TTG TGC CTG TCC TGG GAG AGA CCG GCG CAC AGA GGA AGA GAA TCT CCG CAA GAA AGG GGA GCC TCA CCA CGA GCT GCC CCC AGG GAG CAC TAA GCG AG-3'. The 32-base highlighted with yellow in the sequence was complementary with probe DNA.

S2 was obtained from HCT116 cells, which were cultured in RPMI 1640 medium containing 10% fetal calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin in 5% CO_2 at 37 °C. Genomic DNA was extracted from cells using a Genomic DNA Purification Kit (Promega) according to the manufacture's instruction. DNA concentration and quality were estimated by measuring the absorbance at 260 and 280 nm.

2. Deposition of AuNPs on GC electrode surface.

A glassy carbon electrode (3-mm in diameter, CH Instruments) was used as substrate for deposition of AuNPs. Prior to use, the electrode was polished sequentially with metallographic abrasive paper (No. 6) and slurries of 0.3 and 0.05 μm alumina to create a mirror finish, and then sonicated with absolute ethanol and double distilled water for about 1 min, respectively. It was rinsed thoroughly with double distilled water and dried under ambient temperature. The AuNPs was electrodeposited on the GC electrode surface from aqueous solution of 1 M H_2SO_4 containing 1 mM $AuCl_4^-$ at a constant potential of –0.4 V (versus SCE) for 10 min (step (a) in Fig. 1). Before deposition, the solution was deaerated by purging high-purity nitrogen, and the nitrogen environment was then kept over the solution to prevent oxygen from reaching the solution. After thoroughly rinsed with double distilled water, the AuNPs-covered GC electrode was ready to be used for assembly of DNA. The SEM image of deposited AuNPs and the voltammetric characteristics are presented in Fig. S1 and S2, respectively.

The AuNPs formed on GC electrode surface have an average size of ca. 25 nm. The real surface area of the AuNPs was estimated based on the amount of charge consumed during the reduction of the Au surface oxide monolayer in 1 M H_2SO_4 and a reported value of ca. 400 μC/cm^2 was used for the calculation.[1,2] A value of (0.34 ± 0.03) cm^2, which was an average value of five independent measurements, was obtained in our experimental conditions. The large surface area of the AuNPs will significantly enhance the voltammetric signals.

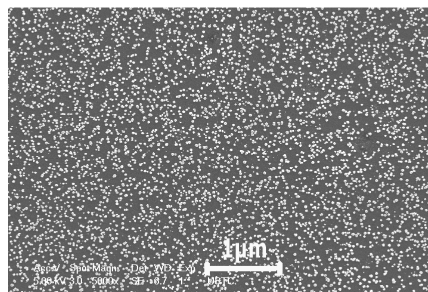

Fig. S1. Typical SEM images of the AuNPs electrochemically deposited on the surface of GC electrode surface under a constant potential of –0.4 V for 10 min in 1 M H_2SO_4 solution containing 1 mM $HAuCl_4$.

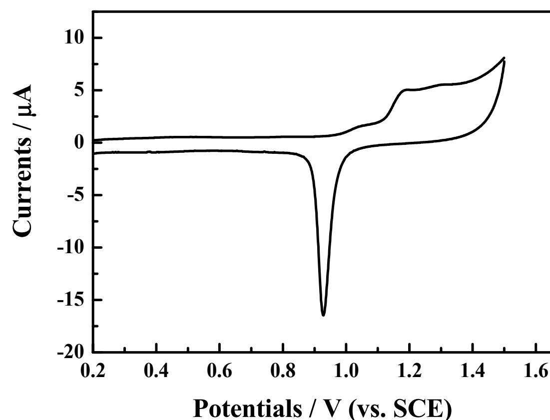

Fig. S2. Cyclic voltammograms of the AuNPs-covered GC electrode in N_2-saturated H_2SO_4 solution (1 M). Scan rate is 10 mV/s.

3. Assembly of S1 on AuNPs-covered GC electrode and hybridization.

For the assembly of S1 on the AuNPs surface, a mixture of 10 μL of S1 solution (10 μM) with 10 μL of TCEP solution (10 mM) was first incubated for 1 h to reduce the disulfide bond at the 5'-terminus of S1 and generate a free thiol group for surface immobilization,[3] followed by diluting the mixture to 100 μL with 10 mM Tris-HCl buffer (pH 7.4). The S1 was immobilized onto the surface of AuNPs surface by incubating the AuNPs-covered GC electrode in the diluted S1 solution for 24 h (step (b) in Fig. 1). The electrode was thoroughly rinsed with Tris-HCl buffer and water in turn, and stored in the buffer.

The redox label of FcA was conjugated to electrode surface by formation the amide between the group of –COOH at FcA and $–NH_2$ moiety at 3'–terminus of the assembled S1 via the succinimide coupling (EDC-NHS). First, the FcA was activated in the EDC-NHS mixture (5 mM EDC, 10 mM NHS in Tris-HCl buffer, pH 7.4) for 1 h. Then the S1-assembled electrode was immersed into the mixture containing 0.5 mM FcA for 5 h (step (c) in Fig. 1). The $–NH_2$ group at S1 would react with the activated –COOH moiety, resulting to covalently link FcA at the 3'-terminus of S1.

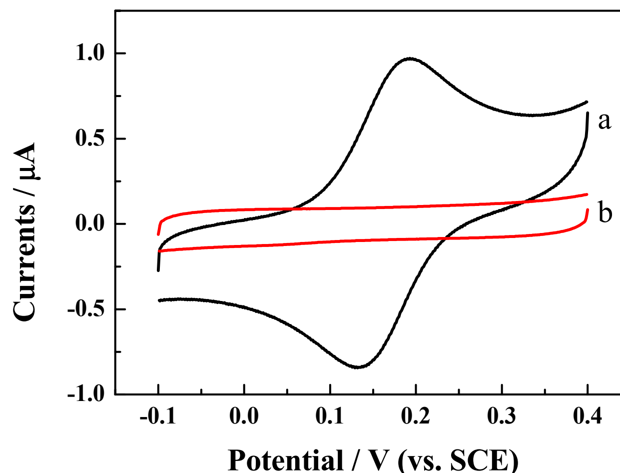

Fig. S3. Cyclic voltammograms of the S1-modified AuNPs-covered GC electrode in 0.1 M acetate buffer (pH 5.0) before (curve b) and after 5-h conjugation with FcA (curve a). The scan rate is 50 mV/s.

Voltammetric results indicated that a pair of well-defined redox peaks with E_{pc} and E_{pa} of ca.140 and 180 mV, respectively, is observed for FcA-S1 (curve b in Fig. S3). The formal potential (E^{0}'), defined as the average of E_{pc} and E_{pa}, is found to be ca.160 mV (vs. SCE), which is similar to those reported by Anne et al. (ca. 170 mV, vs. SCE)[4] and Radi et al. (165 mV, vs. Ag/AgCl)[5] for the same label. Integration of the area of the cyclic voltammetric peaks (such as those presented in Fig. S3) under either the anodic or the cathodic peak, corrected from the background current and measured at a slow scan rate, gives the Faradaic charge required for the full oxidation (or full reduction) of the conjugated FcA. The total amount of bound FcA on the electrode surface (Γ) can be deduced quantitatively from $\Gamma = Q / nFA$, where n is the number of electrons transferred (n = 1 for redox couple of FcA/FcA^{+} in this work), F the Faraday constant, Q the peak area of the immobilized layer in coulombs, and A is the effective surface area of gold electrode (in cm^{2}), thus yielding $\Gamma = (2.96 \pm 0.64) \times 10^{-11}$ mol/cm^{2}, which is equivalent to surface density of $(1.78 \pm 0.39) \times 10^{13}$ FcA molecules/cm^{2} (an average value of five independent measurements). Such a surface density of FcA is close to those values, within the experimental error, reported for the self–assembled monolayer of single–stranded oligonucleotides via sulfur–gold affinity by Radi et al. (ca. 2.25×10^{13} molecules/cm^{2}),[6] Liao et al. ($(1.14 \pm 0.07) \times 10^{13}$

molecules/cm^2),[7] Steel at al. (ca. 1×10^{13} molecules/cm^2),[8] and Zhang et al. (5.1×10^{13} molecules/cm^2).[9] This result suggests that almost every S1 molecule immobilized on the surface of gold electrode has been conjugated with one FcA molecule, demonstrating that the conjugation of FcA with S1 is effective and high efficiency.

Hybridization was conducted at 37 °C by immersing the FcA-S1-assembled electrode in 2 mL of 10 mM Tris-HCl buffer (pH 7.4) containing S2 (0.1 μM) or one-base mismatched DNA (S3, 0.1 μM) for 2 h (step (d) in Fig. 1). After hybridization, the electrode was thoroughly rinsed with water.

4. Methylation of CpG and Inhibition.

The methylation S1/S2 hybrid was performed at 37 °C for 2 h in 10 mM Tris-HCl buffer (pH 7.9) containing 160 μM SAM, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), and various concentration of M. *Sss*I (from 0 to 500 U/mL) (Step (e) in Fig. 1).

To study the inhibition effects of two representative anticancer drugs, 5-Aza and 5-Aza-dC, on the M. *Sss*I activity, the methylation of S1/S2 hybrid was performed at 37 °C in 10 mM Tris-HCl buffer (pH 7.9) containing 160 μM SAM, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 500 U/mL M. *Sss*I, and various concentration of the inhibitors (from 0 to 5 μM).

5. Cleavage of *Hpa*II endonuclease.

*Hpa*II cleavage was performed at 37 ºC in 10 mM Tris-HCl buffer (pH 7.4) containing 20 U/mL *Hpa*II, 50 mM NaCl, 0.1 mM EDTA, 1mM dithiothreitol (DTT), 200 μg/mL bovine serum albumin (BSA), and 50% glycerol for 2 h (step (f) in Fig. 1). After cleavage, the electrode was thoroughly washed and transferred into acetate buffer (0.1 M, pH 5) to record the electrochemical response (step (g) in Fig. 1).

6. Apparatus.

The cyclic voltammetry (CV) and the differential pulse voltammetry (DPV) were employed to sense the covalently bonding of FcA with S1, and to characterize the hybridization and cleavage efficiency since DPV technique has much higher sensitivity than conventional sweep techniques when detecting very low concentrations of redox probe.[10,11] This is achieved by applying a small voltage pulse superimposed on the linear voltage sweep and sampling the differential current at a short time after the pulse. CV and DPV experiments were performed with a CHI 660B electrochemical workstation (CH Instruments). A two–compartment three-electrode cell with a sample volume of 5 mL was employed. A coiled Pt wire and a saturated calomel electrode (SCE) were used as the counter and the reference electrode, respectively. The buffer was purged with high-purity nitrogen for at least 30 min prior to each electrochemical measurement and the nitrogen environment was then kept over the solution to prevent oxygen from reaching the solution. DPV signals were measured using a potential step of 5 mV, pulse width of 25 ms, pulse period of 100 ms, and pulse amplitude of 50 mV.

7. Dependence of DPV signals of FcA on the methylation time.

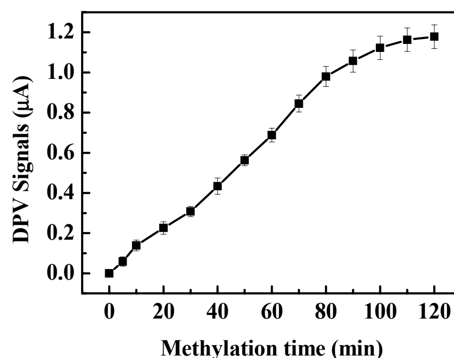

Fig. S4. Dependence of DPV signals of FcA on the methylation time.

8. The inhibition of two typical anticancer drugs 5-Aza and 5-Aza-dC on the activity of M. *Sss*I.

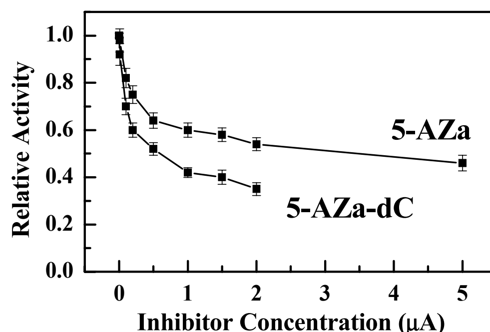

Fig. S5. The dose-dependent inhibition of the activity of M. *Sss*I by two typical anticancer drugs 5-Aza and 5-Aza-dC. The relative activity of the M. *Sss*I was represented as the ratio of the DPV signal of FcA label conjugated to S1/S2 hybrids, which were methylated by M. *Sss*I under various concentrations of inhibitors to that without inhibitors. Before the DPV was recorded, the FcA-S1/S2 hybrids were methylated for 2 h by M. *Sss*I (500 U/mL) at different concentrations of 5-Aza or 5-Aza-dC and then cleaved by *Hpa*II (20 U/mL) for 2 h. Every point is an average value of five measurements.

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